Chapter 5

Discussion
DISCUSSION

The difference in performance in cultivars of periwinkle could be ascribed to the variation in their genetic makeup. The cultivar variations in respect of different parameters have earlier been reported (Virk et al., 1989; Singh et al., 1992; Mishra et al., 2001; Choudhary and Gupta, 2002). Cultivar variations in *Catharanthus roseus* L. on the basis of their morphological traits and in the alkaloid contents that are regulated primarily at the level of gene expression, which is again controlled by the expression of transcription factors was observed by Endt et al. (2002) and Datta et al. (2005).

The genetic potential of the plants is realized to a great extent by the recognized group of chemicals called phytohormones or plant growth regulators. They get involved through the modification of transcription, translation and/or differential sensitivity of the tissue. Firi (1986) has, therefore, suggested that the hormone induced response largely depends on the physiological state of the plant.

Nitrate reducing power of the plant is one of the important factors determining growth. However, the process of nitrate reduction is directly or indirectly dependent on the metabolic sensors and/or signal transducers (Campbell, 1999). The level of the enzyme is dependent on a number of factors, borned within or outside the plants. One of the major regulatory factor, determining the activity of NR, is the level of phytohormones *per se* or that of the ones added from outside (Prakash and Kapoor, 1984; Khan et al., 1996; Ahmad et al., 2001). Brassinosteroids, like other plant growth regulators, act as higher inducer of NR activity (Mai et al., 1989; Singh et al., 1993; Hayat et al., 2001). The present observation (Table 12) is very much comparable with the earlier findings conducted in this regard. Here the leaves of *Catharanthus roseus* L. treated with various PGRs, possessed greater NR activity compared to control.
The level of major electrolyte nitrogen, was affected significantly by growth hormones like IAA, IBA, etc. (Nandwal et al., 1981; Basak et al., 1995). GA₃ highly affected N and K uptake ( Table 13, 15) is in the agreement with the findings of Stefano et al. (1998), Luis and Guardiola (1981) and Hegazi et al. (1995).

The other enzyme, carbonic anhydrase (CA) which catalyzes the reversible hydration of CO₂ and maintains its constant supply to RuBP Case at the level of the grana of the chloroplast (Majeau and Coleman, 1994; Price et al., 1994). Moreover, CA is also known to be involved in photosynthetic electron transport system (Stemler, 1997) and in maintaining chloroplast pH during rapid changes in light intensity (Reed and Graham, 1981). The observed elevation in the activity of CA (Table 11) by different plant growth regulators, specially by HBR, is in the agreement with others (Sugiharto et al., 1992; Khan et al., 1996; Ahmad et al., 2001; Fariduddin et al., 2004). Besides, BR also involves in transcription and/or translation in generating an impact on the activity of CA (Okabe et al., 1980).

Van Staden et al. (1988) proposed that cytokinins boosted the general metabolism of the chloroplast through their action on the related processes operative at the level of the nucleus and/or cytoplasm, facilitating an increase in chloroplast DNA, rate of protein synthesis in the chloroplast, maintaining sufficient pigment level and promotes grana formation. Therefore, leaves of the plants supplemented with cytokinins possessed a great quantity of chlorophyll compared to those sprayed with other plant growth regulators. The application of SA and HBR generated a level of leaf chlorophyll that was comparable to that produced by the application of KIN (Table 8). The effect of SA (Khan et al., 2003; Khodary, 2004) and HBR (Kaur, 1997; Hayat et al., 2000) have also been reported earlier. This improved metabolic state of the treated leaves was further reflected in the observed increase in the net photosynthetic rate (Pₙ) of the leaves (Table 10). Here, the treatment KIN proved to be more effective in comparison to SA and HBR. Kinetin induced activation of gene expression, an
increase in the rate of gene specific transcription and/or an increase in the CA mRNA stability was the findings of Sugiharto and Sugiyama (1992). Furthermore, the KIN implicated environmental responses of plants, including leaf senescence, chloroplast biogenesis, leaf area expansion and dry matter production was observed by Faiss et al. (1997), Hutchinson and Kieber (2002) and Shah (2007).

The leaves of the treated plants possessed a greater surface area (Table 7) which could presumably be due to cell division and cell enlargement induced by the application of TRIA, KIN, HBR and auxins (Eriksen et al., 1981; Nakajima et al., 1996; Arteca, 1997; Clouse and Sasse, 1998). It was further supported by findings of Sairam (1994), Diz et al. (1995) and Khan et al. (1996) where the hormonal treatments increased leaf area. Moreover, the sprayed leaves also exhibited a higher state of metabolic activity. It was evident by the elevated level of net photosynthetic rate that might positively contribute to the enhanced leaf area and plant dry mass production. The superior dry and/or fresh mass of plant shoot and root in response to plant growth regulators treatment could be due to the cumulative effect of enhanced values of various growth parameters. In present growth observations (Table 3-6), the effect of HBR was best among all treatments. brassinosteroids (BRs) are now recognized as an essential regulator of plant growth and development (Li and Chory, 1999) because, their exogenous application restored the normal growth in BR deficit mutant of Arabidopsis (Altman, 1999). The role of BRs in plant growth, and the interaction of BRs with other growth hormones have also been observed earlier (Fujioka and Yokota, 2003; Nomura and Bishop, 2006; Li and Jin, 2007). The two auxins (IAA and IBA) proved their superiority, over GA3 in root enlargement (Table 1, 2). It may be because of many reasons, including the direct involvement of the auxins in cell division and their elongation (Nandwal and Bharti, 1982; Haque, 1989) and/or increased water uptake (Martin and Northcote, 1982). In the present study, the root length was favoured by the auxins, whereas, GA3 increased shoot length. This is in agreement
with the observations reported earlier regarding several crops (Mishirky et al., 1990; Rehman et al., 1994; Dhankar and Singh, 1996; Wynne and Mc Donald, 2002; Tian, 2008).

Significant increases in pod number, seed per pod and seed yield were observed in plants exposed to various plant growth regulators. Improved yield of several important food crops due to TRIA application has been reported by other workers also (Ries, 1985; Kawashima et al., 1987; Kumaravelu et al., 2000; Khan et al., 2006; Khan et al. 2007). The abortion of flowers and pods is one of the important determining factors of plant productivity, where cytokinins are implicated to rescue them from pre-mature fall (Reese et al., 1995; Nagel et al., 2001). The ameliorative effect of cytokinins is, therefore, expected that resulted in increased seed and pod number (Table 16, 17). In the present study, the effect of TRIA application improved the values of yield parameters could have been on comparable lines with that of BAP (Table 16-19). Moreover, higher rate of photosynthesis in the treated leaves ensured regular supply of photosynthates, at an elevated level and availability of more and more organic nitrogen and high protein level provided additional support to the belief that the metabolic state of the leaf is one of the important parameters to evaluate the biological yield (Sairam, 1994).

The biomass production and the accumulation of alkaloids are entirely separate metabolic processes. The biomass production depends on photosynthetic efficiency and/or other factors. The regarding parameters were inhibited by GA3 treatment in the present study (Table 3, 5, 7, 8, 10). Interestingly, in the same study GA3 proved best among PGRs for highest alkaloid accumulation in leaves and roots (Table 20, 21). The same finding was also reported by Srivastava and Srivastava (2007). Distribution and accumulation of alkaloid content in plant parts in Catharanthus roseus vary in roots, stems, and leaves (Misra and Kumar, 2000). There is an imperative associator between inter-organs assimilates transport, particularly shoot and root partitioning of metabolites, and biomass production (Walch-Liu et al.,
How distribution of these metabolites influences alkaloid production by providing organ specific precursor remains to be elucidated (Verpoorte et al., 1997, 1999). A number of sugar, amino acid, and organic acids may act as signals for alkaloid production and possibly biotransformations (Aerts et al., 1996).

**Conclusions**

The present study revealed:

(I) The cultivar 'rosea' gave better response than the cultivar 'alba' to the application of aqueous solution of plant growth regulators.

(ii) Among PGRs, the treatment HBR proved optimum for the production of plant matter. Incomparably, KIN, SA and HBR exhibited a vital influence on most of the physiological and biological parameters.

(iii) Triacontanol (TRIA) proved superior in its effect compared to BAP and other growth hormones for better yield. Whereas, GA₃ treated plants gave best response regarding alkaloid production.

(iv) Hence, on the basis of present observations, interaction HBR × 'rosea' and GA₃ × 'rosea' were optimum to produce maximum plant biomass and total alkaloid content.